5-iodo-4-thio-2-deoxyuridine: synthesis, structure, and cytotoxic activity

Journal Item

5-Iodo-4-thio-2′-deoxyuridine: Synthesis, Structure, and Cytotoxic Activity

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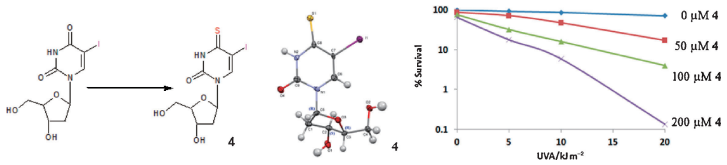

The novel nucleoside 5-iodo-4-thio-2′-deoxyuridine (**4**) was synthesized and fully characterized by IR, NMR, and MS. Its structure was determined by single-crystal X-ray diffraction. Compound **4** absorbs strongly at 346 nm and is minimally toxic to human tumour cells, but its cytotoxicity is substantially enhanced by low dose UVA radiation. The combined use of **4** and UVA offers a promising route to selectively and effectively kill proliferating cells.

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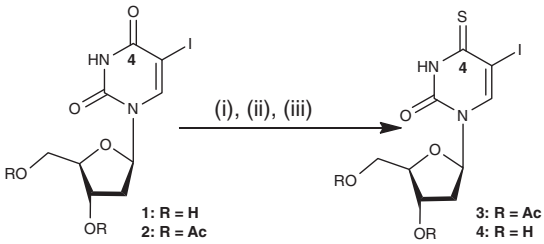
The novel nucleoside 5-iodo-4-thio-2′-deoxyuridine (**4**) was synthesized and fully characterized by IR, NMR, and MS. Its structure was determined by single-crystal X-ray diffraction. Compound **4** absorbs strongly at 346 nm and is minimally toxic to human tumour cells, but its cytotoxicity is substantially enhanced by low dose UVA radiation. The combined use of **4** and UVA offers a promising route to selectively and effectively kill proliferating cells.

Nucleic acids (DNA and RNA) direct the flow of genetic information. In addition to the four canonical bases, structurally similar base analogues can be incorporated into DNA. These alter its properties and facilitate basic studies of DNA-related processes such as its interaction with proteins, replication, and transcription. Site-specifically modified bases and nucleosides are particularly useful in this regard, and thiobases, in which an oxygen atom in the base is replaced by sulfur,[1–3] have proved invaluable in studies of DNA duplex stability, protein recognition, and replication.[4–6] We have been exploring the photochemical properties of thiobases and their derivatives, particularly 6-thioguanine (6-TG) and 4-thiothymidine (4-thioTdR). Unlike canonical DNA bases, both are strong UVA chromophores and are extremely sensitive to wavelengths of 315–400 nm (UVA range). Upon exposure to UVA radiation, DNA-embedded 6-thioguanine is oxidized to guanine-6-sulfinate and guanine-6-sulfonate in an oxygen-dependent reaction.[7–9] UVA irradiation of cells containing DNA 6-TG also induces DNA strand breakage and interstrand crosslinking.[10] These DNA lesions block replication and transcription and cause cell death.[11] DNA 4-thioTdR also displays synergistic lethality with UVA radiation. It undergoes UVA-mediated conversion to potentially cytotoxic lesions including inter- and intrastrand DNA crosslinks.[12] In the absence of UVA, cells can accumulate significant levels of 4-thioTdR without detrimental effects on proliferation.[13,14] The combined use of 4-thioTdR and UVA offers a novel approach to selectively kill rapidly proliferating cells, such as cancer cells, while causing minimal damage to normal tissues.[15] The advantage this synergistic photoactivation of DNA thiobases has over conventional therapies is that it can target proliferating cells more selectively. We have therefore explored the properties of additional 4-thioTdR analogues and have previously reported the synthesis and chemical properties of 5-bromo-4-thio-2′-deoxyuridine.[16] Here we report a synthetic route for 5-iodo-4-thio-2′-deoxyuridine. We also describe its crystal structure and initial studies of its cytotoxicity.



Scheme 1. Synthesis of 5-iodo-4-thio-2′-deoxyuridine (**4**). (i) Ac_2O/pyridine (91% for **2**); (ii) P_4S_{10}/1,4-dioxane (74% for **3**); (iii) NH_3(g)/MeOH (68% for **4**).

Table 1. Key spectral data of compounds **1** and **4**

Cpd	UV (λ_{max}/nm)	IR /cm^{-1}	^{1}H NMR (N3–H)/ppm	^{13}C NMR (C4)/ppm
1	290	1677 (C4=O)	11.66	160.48
4	346	1088 (C4=S)	12.99	189.31

The synthetic route is shown in Scheme 1 in which the starting material **1** was first acetylated at its 3′- and 5′-positions to give the acetylated nucleoside **2**, followed by a thiolation at the 4-postion to give the protected 4-thio-nucleoside **3**. This was then deprotected to afford the target product **4**. Previously we used triazole/$POCl_3$ and thioacetic acid to produce **3**.[17a] The current synthesis is straightforward and proceeds with good yields (see ESI for details). The intermediates **2** and **3** and the product **4** were fully characterized by MS, UV, IR spectra, ^{1}H NMR and ^{13}C NMR measurements (see ESI for details). Some key spectral data of **1** and **4** are summarized in Table 1 for comparison.

The spectral characterization provides a good confirmation for the structure of **4**. High-resolution MS analysis of **4** gave an ion at 392.9382 for the molecular fragment ($M + Na^+$), the m/z being within 1 ppm of the calculated m/z (392.9378) for $C_9H_{11}IN_2NaO_4S$. **4** absorbs strongly at 346 nm, substantially shifted from the 290 nm maximum of **1** (Figure S1, see ESI). This red shift reflects the presence of the sulfur atom.[16] The observed band at 1088 cm^{-1} in the infrared spectrum is within the characteristic range (1070–1150 cm^{-1}) assigned to the C=S stretching mode of the thioamide group in 4-thiouridine,[18] indicating the presence of the C4=S group in **4**. NMR data offer further support. D_2O exchange experiments readily identify the signal in δ_H 12.99 ppm as proton N3–H. The presence of the thioamide in **4** is also evident from the substantial NMR

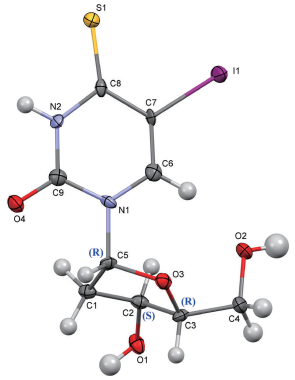


Figure 1. Molecular structure and absolute configuration of **4**. Only one independent molecule present in the crystal structure is shown. Full structural data are deposited at the Cambridge Crystallographic Data Centre, depository number: 959345.

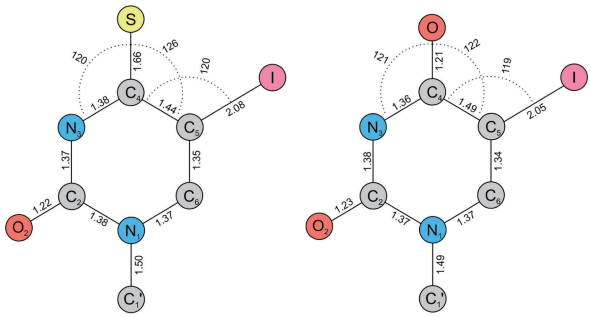


Figure 2. Selected bond lengths (Å) and angles (°) of the bases in **4** and **1**. For compound **4** the average values of cell bond distances and angles of three independent molecules present in the asymmetric part of the unit are shown.

chemical shifts[17] of N3–H (12.99 from 11.66) and of C4 (189.31 from 160.48) (Table 1).

The molecular structure and absolute configuration of **4** (Figure 1) was confirmed by single-crystal X-ray diffraction analysis.

The structure belongs to the triclinic system and space group *P*1 (Table S1, see ESI). It is worth noting that the unit cell of the crystal structure of 5-iodo-4-thio-2′-deoxyuridine contains three independent molecules (Figure S2, see ESI). It is not unusual to have two independent molecules of a nucleoside in a single unit cell, for example deoxyuridine and 5-bromo-2′-deoxyuridine,[19] but it is rare for the same unit cell to contain three or more nucleosides.[20]

Figure 2 shows the comparison of the lengths and angles of individual bonds in the bases **4** (5-iodo-4-thio-2′-deoxyuridine) and **1** (5-iodo-2′-deoxyuridine).[21] Overall, these two molecular structures are very similar, except the bond involving the sulfur atom at the 4-position. The thiocarbonyl (C4=S) bond in **4** is substantially longer than the carbonyl (C4=O) bond in **1**. The average length (from three individual molecules of **4**) of C4=S bond is 1.662 Å and is similar to that of the documented C=S bond of 4-thiouracil derivatives (1.672 Å) and the mean C=S distance (1.654–1.665 Å) reported by Allen et al.[22] This length is much shorter than a standard C–S single bond of 1.80 Å, demonstrating that the thiocarbonyl group is in fact a double bond. The angle ∠SC4C5 (126°) is slightly wider than the angle ∠OC4C5 (122°), and the C4=S bond tilts away from the iodo group at the 5-position.

In the crystal lattice each of three independent molecules are linked together by an intermolecular hydrogen-bond network (Figure S3, see ESI). Each independent molecule serves as a donor and acceptor of moderate O–H···O (D···A ≈ 2.765 Å) and N–H···O (D···A ≈ 2.285 Å) type hydrogen bonds. Additionally, each sulfur atom is involved in a weak O–H···S (D···A ≈ 3.394 Å) hydrogen bond (Table S2). The C=S bond is known to have a broader distance range and to be less polar than a $C^{\delta+}=O^{\delta-}$ bond, thus it is also a weaker hydrogen bond acceptor. For C=S acceptors, the mean hydrogen bond distances are more than 0.5 Å longer than for C=O acceptors. This is also larger than the difference of the S and O van der Waals radii and the lone-pair directionality at C=S acceptors is very pronounced.[22,23] Interestingly, systematic studies by Allen et al.[22] showed that only the longer C=S bonds appear to be involved in hydrogen bonding (the percentage of hydrogen bonds formed by C=S increases systematically from 5% to 75% as the C=S bond length increases from 1.63 to 1.75 Å). Detailed bond lengths and angles for the three individual molecules present in the crystal structure of **4** are available from the CCDC, depository number: 959345.

5-Iodo-2′-deoxyuridine (**1**) was one of the first selective antiviral nucleosides.[24] It is still used in the treatment of various diseases including herpetic keratitis[25] and for radiosensitization of high-grade gliomas.[26] Previously we showed 4-thioTdR has very similar base-pairing properties to thymidine.[14] As **4** and **1** differ only slightly in structure, it is highly likely that their behavior would be similar in terms of incorporation into viral DNA and base-pairing. However, the strong UVA absorption of **4** would enhance its potential as an UVA-mediated antiviral agent or DNA probe. We therefore examined the effects of photochemical activation of **4** on human cells growing in culture.

Compound **4** was toxic to cultured human cells only at high μM concentrations. Figure 3 shows the effects of compound **4** on the viability of the human oral cell carcinoma CA-1 cells. Continuous 24 h exposure induced a dose-dependent increase in cytotoxicity with an approximate LD_{50} of 320 μM. Clearly **4** was minimally toxic to the cultured human cells.

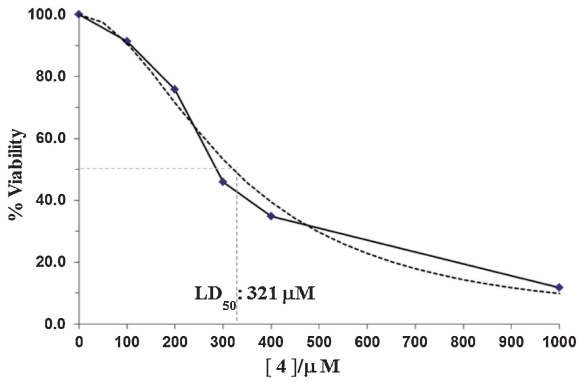


Figure 3. Dose-dependent cytotoxicity of **4** in human oral cell carcinoma CA-1 cells. The solid line is the experimental data and the dotted line is a fitted curve.

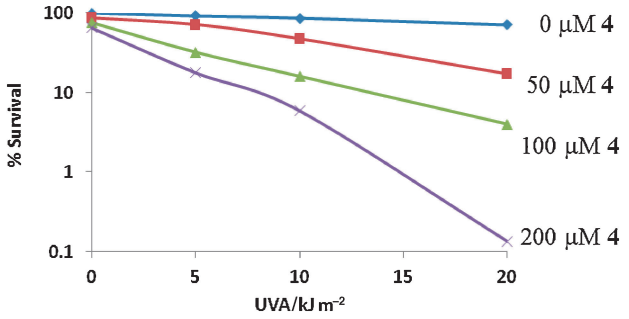


Figure 4. UVA sensitization by compound **4** in HEK cells. Cells were treated in growth medium with compound **4** for 48 h at the concentrations shown (see ESI for details).

The toxicity of **4** towards human HEK293 embryonic kidney cells was similar and treatment with 100 or 200 μM for 48 h reduced survival by 10–20% (Figure 4). Direct analysis of DNA from treated cells confirmed a dose-dependent accumulation of **4** in DNA. The presence of DNA-embedded **4** was associated with a significant sensitization to nontoxic dose of UVA (340 nm) (Figure 4).

UVA doses up to 20 kJ m^{-2} caused only ≤10% toxicity in the absence of drug pretreatment, but cell survival was significantly reduced after combined compound **4**/UVA treatment in both a UVA-dependent and compound **4**-dependent fashion. At the highest drug concentration (200 μM) and 20 kJ m^{-2}, UVA reduced cell viability by >99.5%. These preliminary results suggest that 5-iodo-4-thio-2′-deoxyuridine in combination with UVA is more cytotoxic than 5-bromo-4-thio-2′-deoxyuridine.[16] This increased cytotoxicity of **4** might reflect a lower bond enthalpy for the C–I bond[27] (e.g. DH_{298} = 241 kJ mol^{-1} for CH_3–I) compared to that for C–Br bond (e.g. DH_{298} = 302 kJ mol^{-1} for CH_3–Br), suggesting 5-iodo-4-thio-2′-deoxyuridine would be more susceptible to UVA-induced activation than 5-bromo-4-thiodeoxyuridine.[16] Studies are ongoing to define the nature of the lethal DNA lesions and the mechanism of cytotoxicity of the 5-iodo-4-thio-2′-deoxyuridine/UVA combination.

In conclusion, a UVA-sensitive nucleoside, 5-iodo-4-thio-2′-deoxyuridine, has been synthesized in a good yield and fully characterized by spectroscopy. A single-crystal structure was also obtained by X-ray analysis. The thio-nucleoside was incorporated effectively into DNA of cultured human cells. This caused a modest antiproliferative effect that was greatly enhanced by low doses of UVA radiation. The combined use of 5-iodo-4-thio-2′-deoxyuridine and UVA offers a promising route to selectively and effectively kill proliferating cells.

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Supporting Information is available electronically on J-STAGE.

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